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Distribution of mtDNA Haplogroup X Among Native North Americans

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ABSTRACT Mitochondrial DNA (mtDNA) samples of 70 Native Americans, most of whom had been found not to belong to any of the four common Native American haplogroups (A, B, C, and D), were analyzed for the presence of Dde I site losses at np 1715 and np 10394. These two mutations are characteristic of haplogroup X which might be of European origin. The first hypervariable segment (HVSI) of the non-coding control region (CR) of mtDNA of a representative selection of samples exhibiting these mutations was sequenced to confirm their assignment to haplogroup X. Thirty-two of the samples exhibited the restriction site losses characteristic of haplogroup X and, when sequenced, a representative selection ($n = 11$) of these exhibited the CR mutations commonly associated with haplogroup X, C → T transitions at np 16278 and 16223, in addition to as many as three other HVSI mutations. The wide distribution of this haplogroup throughout North America, and its prehistoric presence there, are consistent with its being a fifth founding haplogroup exhibited by about 3% of modern Native Americans. Its markedly nonrandom distribution with high frequency in certain regions, as for the other four major mtDNA haplogroups, should facilitate establishing ancestor/descendant relationships between modern and prehistoric groups of Native Americans. The low frequency of haplogroups other than A, B, C, D, and X among the samples studied suggests a paucity of both recent non-Native American maternal admixture in alleged fullblood Native Americans and mutations at the restriction sites that characterize the five haplogroups as well as the absence of additional (undiscovered) founding haplogroups. Am J Phys Anthropol 110:271–284, 1999.

Restriction analysis of mitochondrial DNA (mtDNA) has demonstrated that most Native Americans exhibit one of four mutations identifiable by the gain or loss of a restriction site (haplogroups A, C, and D) or, in the case of haplogroup B, by a 9-bp deletion (Schurr et al., 1990). Subsequent sequencing analysis revealed that each of the four haplogroups is also characterized by at least one unique mutation, or, in the case of haplogroup D, by a typical combination of

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mutations, within the mtDNA (noncoding) control region (CR) (Torroni et al., 1993). These four haplogroups have been argued to represent founding lineages because all four (1) are also found in Asia, presumed to be the homeland of all Native Americans, (2) occupy central nodes in a phylogeny of New World mtDNA haplotypes, and (3) are widespread throughout North, Central and South America.

While widespread, the geographic distribution of the four haplogroups is markedly nonrandom (Lorenz and Smith, 1996). For example, haplogroup A is extremely common among Eskimo/Aleut and Northern Athapaskan tribes, but extremely rare in non-Athapaskan speakers of the Southwest United States. Haplogroup B is extremely common in the American Southwest but absent, or rare, in the Arctic, Subarctic, and Northwest Coastal regions. Haplogroup D, while present throughout the New World, is the least common of the four haplogroups everywhere except in certain Western tribal groups, notably speakers of Penutian languages in whom it represents the most common haplogroup. The mtDNA haplogroup distributions of modern Native American groups are quite uniform among many tribal groups known to be closely related (e.g., see Lorenz and Smith, 1996), suggesting that genetic drift has not influenced their distributions sufficiently to preclude their use for assessing ancestor/descendant relationships between living populations and prehistoric skeletal material (e.g., Kaestle, 1997; Malhi, 1998; Parr et al., 1996; Stone and Stoneking, 1998; Carlyle et al., 1999) in nearby regions.

Other mtDNA haplogroups

The remaining few Native Americans that do not exhibit one of these four haplogroups have been termed "others" (Torroni et al., 1993) or members of "haplogroup E" (Bailliet et al., 1994) and could represent recent non-Native American admixture and/or mutations at the diagnostic restriction site for one of the four common haplogroups (Torroni and Wallace, 1995). Haplotypes that are not members of haplogroups A, B, C, or D and that are characterized by specific mutations have been reported in modern (presumably unmixed) Native Americans (Bailliet et al., 1994) and in prehistoric individuals (Stone and Stoneking, 1993; 1998; Ribeiro-dos-Santos et al., 1996; Kaestle, 1997), suggesting that founding haplogroups other than A, B, C, and D were once present in the New World, whether or not they have survived.

Bailliet et al. (1994) argued that the Hae III site at np 16517 (cited above) characterizes two subtypes of each of three of the four major haplogroups (A, C, and D), each subtype representing two different founding lineages associated with each of the three haplogroups. Alternatively, Torroni et al. (1993) have argued that the np 16517 site is hypervariable and, therefore, that the emergence of parallel subtypes of founding haplogroups in the New World post-dates the settlement of the New World. Bailliet et al. (1994) rejected this argument because it could not explain the apparent absence of any members of haplogroup B who also exhibit a *Hae* III site loss at np 16517. However, Easton et al. (1996) later reported that this mutation characterizes two subtypes of haplogroup B in the Yanomami.

Easton et al. (1996) reported that haplogroups they called X6 and X7 comprised about 12% of the Yanomami samples they studied. These are found widespread in the New World and have been reported in Asia as well (Merriwether and Ferrell, 1996). Members of these haplogroups share most of the CR mutations, but lack the restriction sites (e.g., gains of a *Dde* I and an *Alu* I restriction site at np 10394 and np 10397, respectively), characteristic of haplogroups C and D and differ from each other by the presence or absence of the *Hae* III restriction site at np 16517. However, there are numerous apparent errors in those published sequences (e.g., half of all mutations reported were transversions) and the CR sequences of members of haplogroups X6 and X7 always cluster with members of haplogroup C and/or haplogroup D of which they are probably reversions (Stone and Stoneking, 1998). Since the *Hae* III restriction site at np 16517 appears to be a hypervariable site that might have experienced numerous transitions followed by reversions, it is not clear whether or not X6 and

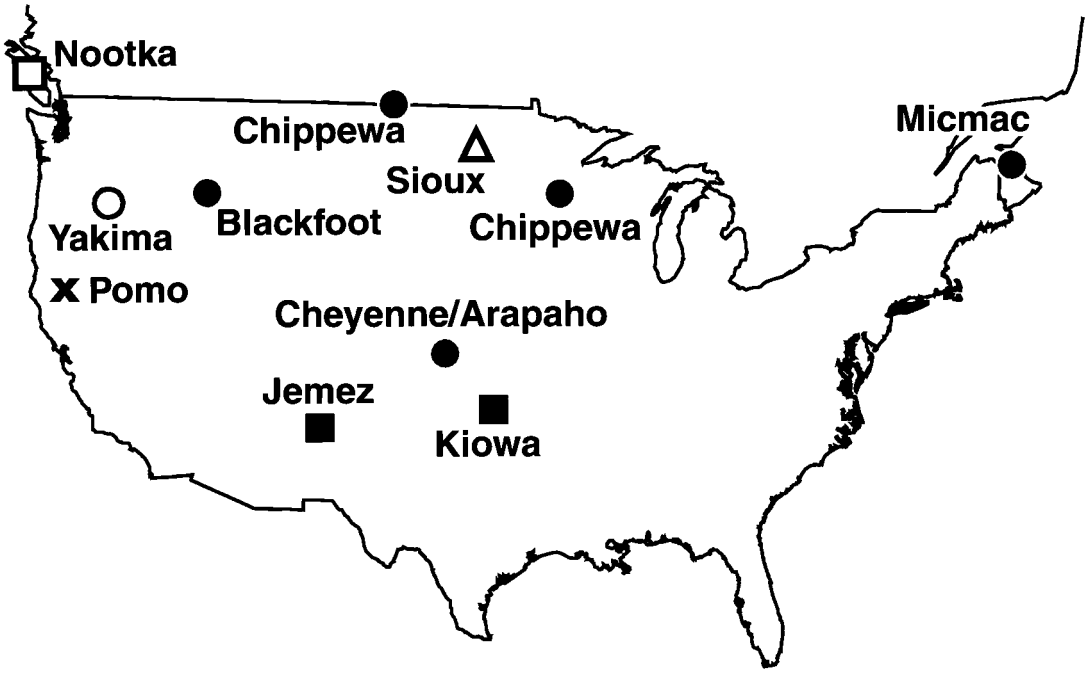

Fig. 1. Distribution of Native American tribes assigned to haplogroup X. The language group of each tribe is coded as follows: Algonquian, ●; Kiowa-Tanoan, ■; Wakashan, □; Penutian, ○; Northern Hokan, ×; Siouan, △.

X7 are founding haplogroups. That site np 16517 is hypervariable is more plausible than is the random survival of exactly two representatives that are mutually distinguishable by the same mutation in each of four (or five) already rare Asian lineages.

Forster et al. (1996) have argued that two variants of haplogroup A (differentiated by the presence or absence of a C → T transition at np 16111) comprise two separate founding lineages. Given recent evidence for a single New World migration (e.g., see Merriwether et al., 1995; Lorenz and Smith, 1997; Stone and Stoneking, 1998; Bonatto and Salzano, 1997a,b), the presence of both variants in Amerind, Eskimo-Aleut and Na-Dene populations (Shields et al., 1993), whom many once regarded as descendants of at least two, if not three, separate migrations to the New World, does not conflict with this hypothesis. However, the absence of this mutation is fairly rare, is concentrated in the Northwest of the continent, and might represent a reversion of New World origin followed by admixture among unrelated groups. Thus, none of the above-cited candidates for an additional (i.e., fifth) Native American founding mtDNA lineage is supported by compelling evidence or satisfies the three criteria Torroni et al. (1993) cited as being sufficient and necessary to constitute evidence for a founding lineage.

Is there a fifth mtDNA lineage in North America?

Bailliet et al. (1994) suggested the possibility of a fifth haplogroup, defined by a C → T transition at np 16278 and the absence of the mutations that characterize haplogroups A, B, C or D. This haplogroup is the same as that comprising the Nuu-Chah-Nulth (i.e. Nootka) lineage cluster I in the maximum likelihood phylogeny of Ward et al. (1991; figure 2). Forster et al. (1996) considered this haplogroup to be identical to haplogroup X, described by Torroni et al. (1996), which is not to be confused with haplogroups X6 and X7 proposed by Easton et al. (1996). Haplogroup X is also characterized by *Dde* I site losses at np 1715 and np 10394, muta-

tions that are otherwise rare in North America (Brown et al., 1997). The haplogroup occupied a nodal position in a phenogram, one of three criteria cited by Torroni et al. (1993) as necessary and sufficient to constitute a founding haplogroup of the New World. The haplogroup is also one of at least ten found in Europe (at a frequency of about 3%) (Torroni et al., 1996), but not East Asia, and was once, but no longer, assumed to represent European admixture in the New World (Torroni et al., 1993; Scozzari et al., 1997). The C → T transition at np 16278 is also found in Africa, where it typically accompanies a *Dde* I site gain at np 10394 which, in Europe, is found only in members of haplogroups I, J, and K. It is also common in Asian haplotypes, including a minority of those in haplogroup F defined by Kolman et al. (1996), but it is not known whether or not such individuals exhibit the *Dde* I restriction site loss at np 1715. In contrast, the *Dde* I site loss at np 1715, but not the C → T transition at np 16278, is characteristic of Europeans of haplogroup I (and a small subset of members of haplogroup H) but co-occurs with the *Dde* I site gain at np 10394 (Torroni et al., 1994). In the New World, the C → T transition at np 16278 (but no other CR mutations characteristic of haplogroup X) has been reported in a Pima Indian belonging to haplogroup B, a Kraho tribesman (from Brazil) belonging to haplogroup A (Torroni et al., 1993), three Nuu-Chah-Nulth belonging to haplogroup A (Lorenz and Smith, 1997), and a Washo member of haplogroup C (Kaestle, 1998), but is otherwise rare or absent in Native Americans of haplogroups A, B, C, and D in whom it probably represents a mutation independent of that at np 16278 in Native Americans with the *Dde* I site loss at np 1715. This same C → T transition has also been characterized in three of 18 pre-Columbian Amerindian skeletons from South America two of which are about 4,000 years old (Ribeiro-dos-santos et al., 1996); while this mutation alone is not diagnostic of membership in haplogroup X, the authors, like Bailliet et al. (1994), consider it characteristic of a fifth haplogroup, which they call V, that was not among those defined by Horai et al. (1993). In addition, this mutation has been found in two of 16 prehistoric skeletons from Windover Pond, Florida dated to between 7,000 and 8,000 years BP (Hauswirth et al., 1994) and in an approximately 600-year-old Oneota sample from the Norris Farms site (Stone and Stoneking, 1998) and, therefore, is unlikely to represent post-Columbian European admixture. Recently, the CR mutations (C → T transition at np 16223 and C → T transition at np 16278) were reported in an individual from the Los Vaqueros site in Central California, dating to approximately 7,400 ybp (Eshleman, unpublished data). Although the C → T transition at np 16278 is typically associated with the *Dde* I site loss at np 1715 in both North America and Europe, but not elsewhere, restriction analysis of the prehistoric samples from the four archaeological sites described above has not been done, so it is not certain that they are members of the same haplogroup as that reported by Forster et al. (1996).

Forster et al. (1996) reported that seven of 62 Nuu-Chah-Nulth (11%) [with haplotypes similar to those comprising lineage cluster I in Figure 2 of Ward et al. (1991)] and two of 40 Yakima (5%), whose haplotypes occupy central nodes in their phylogeny, exhibited both the C → T transition at np 16278 and the *Dde* I site loss at np 1715 and, therefore, are members of haplogroup X. Although, Stone and Stoneking (1998; Fig. 2) have shown that four of these seven Nuu-Chah-Nulth, as well as their prehistoric Oneota sample that was assigned to the "other" haplogroup, show some similarities to members of the Asian haplogroup F [described by Kolman et al. (1996)], the Oneota sample was not tested for the *Dde* I site loss at np 1715. The hypothesized fifth haplogroup, defined by a *Dde* site loss at np 1715, also represented about one-quarter of the haplogroups assigned by Scozzari et al. (1997) to a group of 63 Native Americans of two different Chippewa (known as Ojibwa in Canada) tribes. Since haplogroup X appears to be absent in both the Dogrib and Tanana (Brown et al., 1998) its presence in the Navaho might result from their admixture with unrelated Pueblo groups (Brown et al., 1958; Lorenz and Smith, 1996) after their arrival in the Southwest approximately half

a millenium ago (Brugge, 1983). Although haplogroup X has not been found in East Asia, the prehistoric linkage between the C → T transition at np 16278 and the *Dde I* restriction site loss at np 1715 in Native America suggests that haplogroup X might be a founding lineage, as Forster et al. (1996) and Brown et al. (1998) have argued, rather than representative of recent European admixture. However, notwithstanding the speculation of Brown et al. (1998), that haplogroup X in North America is limited to Northern Amerindian groups, it is not known whether or not haplogroup X is widely distributed throughout the New World, as would be expected of a founding Native American matriline.

Is haplogroup X a fifth founding lineage?

To assess the likelihood that haplogroup X is a fifth founding lineage, the proportions of haplogroups previously described as "other," which themselves are known to be widely distributed in Native America (Lorenz and Smith, 1996), that actually belong to haplogroup X must be determined and the distribution of this haplogroup in North America characterized, as we have previously done for the other four major founding haplogroups (Lorenz and Smith, 1996). If those "other" haplogroups that prove to be members of haplogroup X are very restricted geographically, occurring, for example, only in the three tribes cited above, they might share, through admixture, a recent mutation that destroyed the characteristic marker of haplogroup A, B, C, or D, and therefore constitute a subtype of one of these four founding lineages. If very few of the "other" haplogroups (while widely dispersed geographically), are identified as X, then an even larger number of founding lineages might exist, and the first immigrants to the New World might have brought a far more representative sample of Asian (or Eurasian) lineages to the New World than has been alleged (Wallace et al., 1985). If, on the other hand, most, or all, of the members of this diverse group of "other" haplogroups are identified as members of haplogroup X, these five haplogroups might represent all the surviving founding New World haplogroups, as Forster et al. (1996) have argued. Since the five New World haplogroups represent a minority of those surviving in the Old World (Bailliet et al., 1994), it is of interest to determine whether or not additional founding haplogroups were once present, but have since become extinct, in the New World. Further studies of ancient DNA, such as those conducted by Stone and Stoneking (1993; 1998), Parr et al. (1996), Kaestle (1997; 1998), Malhi (1998), and Carlyle et al. (1999), would then be required to determine whether a restricted number of haplogroups in the New World reflects a bottleneck or a founder effect in the evolutionary history of Native America. In either event, knowledge of the frequency distribution of this and other less common founding lineages will increase the utility of haplogroup distributions as "signatures" of ancestor/descendant relationships between living and prehistoric (skeletal) populations.

MATERIALS AND METHODS

The samples

We have previously characterized the geographic and ethnic distributions of haplogroups A, B, C, and D in a sample of 829 Native North Americans of alleged unmixed ancestry (Lorenz and Smith, 1996). In that study, we determined that 50 of the 829 individuals (or 6%) lacked the restriction sites, or the 9-bp deletion, that characterize any of these four haplogroups, and were, therefore, described as "other." These 50 samples were geographically dispersed among 9 of the 12 culture areas defined by Driver and Massey (1957) and among 12 of the 26 language groups defined by Campbell and Mithun (1979) that were represented by tribes included in this study. Of these 50, sufficient sample for further study was available for only 25 (data for most of the other 25 samples had been taken from published reports). Eleven of the 25 samples unavailable for further study were Chippewa, and at least one each of the remainder represented six different unrelated tribal groups. For all but four of the ethnic groups exhibiting at least one "other" haplogroup among the 25 (Cora, Maya, Navaho, and Nuu-Chah-Nulth), at least one additional sample remained for study. As described above, the presence of haplogroup X in one of these four

groups unrepresented in the samples available for this study, the Nuu-Chah-Nulth, has already been documented (Forster et al., 1996). The presence of haplogroup X in several Navaho (southern Athapaskan) samples has been reported (haplotype AM 29 described in Torroni et al., 1992 and in Brown et al., 1998) but might result from extensive and recent admixture of Navaho with Pueblo tribes living in the Southwest (Brugge, 1983) that correspondingly exhibit traits common in Athapascans but rare or absent in the Southwest (Brown et al., 1958; Lorenz and Smith, 1996). When the 25 samples were rescreened for the characteristic markers for haplogroups A, B, C and D, seven were discovered to have been mistyped; two each belonged to haplogroups A and C and three belonged to haplogroup D, respectively.

Due to the discovery of seven mistyped samples, we randomly selected 58 of the original 512 samples for DNA sequencing of the HVSI region (Lorenz and Smith, 1996) in order to estimate an error rate in identifying haplogroups through restriction analysis. Using the presence of diagnostic markers in the CR, as described in Lorenz and Smith (1997), samples were identified as members of one of three haplogroups (A, B, or C). Additionally, members of haplogroup A were also identified by the presence of at least 2 of 3 markers in the HVSII portion of the control region: a T → C transition at np 146, an A → G transition at np 153, and an A → G transition at np 235 (Malhi, unpublished data). Of the 58 samples sequenced, 17 were assigned to haplogroup A, 20 to haplogroup B, and 21 to haplogroup C through restriction analysis. All 17 samples assigned to haplogroup A and all 20 samples assigned to haplogroup B contained HVSI or HVSII CR diagnostic markers that agreed with their original haplogroup assignments. Of the 21 samples assigned to haplogroup C by restriction analysis, 20 contained CR markers that agreed with their original haplogroup assignment. The remaining sample assigned to haplogroup C had no CR markers for the any of the five founding haplogroups. The single sample that did not have any diagnostic CR markers was re-haplotyped by restriction analysis and sequence analysis of the HVSI region and gave the same results. Therefore, the disagreement between restriction analysis and sequence analysis of the HVSI region in haplogroup assignment of this one sample was not due to mistyping. We estimate a 1.7% rate of inconsistency between haplogroup assignments based on restriction analysis and sequence analysis of HVSI. Since no mistyping of the 58 samples assigned to haplogroups A, B or C occurred, the higher percentage of mistypes in the "others" category probably occurred because the authors were conservative in assigning samples to each of the four common haplogroups to avoid observer errors. Thus, only 3–4% of the Native American samples originally screened were not members of one of the four major Native American matrilines.

Twenty (of 70) additional samples of sera from Chippewa from the Lac Courte Oreilles community in Hayward, Wisconsin (or Southwestern Ojibwa) that were determined to be members of "other" haplogroups as parts of unrelated studies, and one sample each representing the Pomo, Blackfoot, and Sioux (Kaestle, 1997; Malhi, 1998; Smith et al., 1999) were also included in the present study. A total of 30 individuals from a sample of 189 Salteaux (or Northwestern) Chippewa, not previously screened for haplogroups A, B, C or D, were screened only for the presence of the *Dde* I site losses at np 1715 and 10394 that characterize haplogroup X. Since samples from other Chippewa groups had been found to exhibit this mutation (Scozzari et al., 1997), we studied 30 additional samples from a Salteaux Chippewa group merely to confirm its presence. Finally, our screening of a sample of 118 additional Northern Paiute samples yielded no members of haplogroups other than A, B, C, or D.

Laboratory analyses

Each of these 70 samples, whose ethnic identities and geographical origins are summarized in Table 1, was extracted using methods described in Lorenz and Smith (1996), then amplified using the primer coordinants L1631–1651 and H1793–1776 and

TABLE 1. Samples screened for haplogroup X in this study

Ethnic group	Language group	Number tested	Number assigned to haplogroup X (% of all samples[1])
Southwestern (Lac Courte Oreilles) Chippewa	Algonquian	22[2,7]	18 (25.7)
Northwestern (Salteaux) Chippewa	Algonquian	30[3]	2 (6.7)
Cheyenne/ Arapaho	Algonquian	2	2 (11.1)
Micmac	Algonquian	2	2 (40)
Narragansett	Algonquian	1[4]	0 (0)
Blackfoot	Algonquian	1	1 (100)
Cherokee	Iroquoian	4[2,6]	0 (0)
Kiowa	Kiowa-Tanoan	2	2 (40)
Jemez Pueblo	Kiowa-Tanoan	3	3 (8.1)
Creek	Muskogean	1[5]	0 (0)
Pomo	Northern Hokan	1	1 (25)
Sioux	Siouan	1	1 (100)

[1] Estimate of the frequency of haplogroup X in all samples tested of the ethnic group indicated.
[2] Two of these samples are members of haplogroup H which is of European origin.
[3] These 30 samples are the only ones of the 91 studied that had not previously been determined not to belong to haplogroup A, B, C or D.
[4] Member of haplogroup L.
[5] Probably a member of haplogroup A, based on its CR sequence, that lost the Hae III restriction site at np 633 characteristic of haplogroup A.
[6] One of these samples is probably a member of haplogroup C, based on its CR sequence, that regained the *Hin*c II restriction site loss at np 13259 that is characteristic of haplogroup C; another of these samples is a member of European haplogroup J.
[7] One of these samples is a member of European haplogroup T and an insufficient volume of another of those samples remained for additional restriction analysis.

H10579–10557 and L 10270–10290 that were designed, to our specifications, by Genset (La Jolla, CA). Opportunities for sample contamination were minimized by implementing the appropriate precautions and procedures described in Kaestle (1997; 1998). Amplifications were performed in 25 μl capillary tubes using a 1605 Thermal Cycler (Idaho Technology) and conditions for amplification as described in Lorenz and Smith (1996), except that 45 polymerase chain reaction (PCR) cycles were conducted using an annealing temperature of 50°C.

After confirming successful amplification of PCR fragments of the appropriate size by electrophoresis of 5 μl of the PCR product on a 6% polyacrylamide gel stained with ethidium bromide, the remaining products were digested overnight with 10 units of the *Dde* I restriction enzyme (Boehringer Mannheim). Portions (5–10 μl) of the restriction digests were electrophoresed on a 6% polyacrylamide gel, stained with ethidium bromide, and photographed over a UV transilluminator, using the ISO 2000 imaging system (Alpha Innotech, San Leandro, CA) to determine whether or not the amplified fragments had been cut by the restriction enzyme. Samples both of whose PCR fragments were not successfully digested into two segments of the appropriate size, because they lacked a restriction site at both np 1715 and np 10394, were regarded as members of haplogroup X.

Both strands of the HVSI of the mtDNA CR of a representative subset ($n = 11$) of the "other" samples with the *Dde* I restriction site losses at np 1715 and np 10394 were amplified using PCR primers with coordinants L 16021–16038 and H 16375–16356 (or H 00018–16568 for the Jemez samples). These included the Sioux sample exhibiting albumin Naskapi described in Smith et al. (1999). These 11 samples were submitted for sequencing to the DBS Automated DNA Sequencing Laboratory at University of California, Davis. The sequences were compared with the Anderson sequence (Anderson et al., 1981) and with the published sequences of other samples (e.g., Nuu-Chah-Nulth, Yakima, Chippewa, Navaho) assigned to haplogroup X or exhibiting CR mutations that are characteristic of haplogroup X.

For each of the 70 samples that did not exhibit *Dde* I site losses at both np 1715 and np 10394 the fragments defined by the primer coordinates given in Table 1 (from Torroni et al., 1996) were amplified and digested with the restriction enzymes indicated. Because these restriction sites, together with those characteristic of haplogroups A, B, C, D, and X, characterize haplogroups of between two-thirds and three-quarters of the population in the geographic regions indicated, this screening could reveal the presence of haplotypes acquired through recent admixture. The HVSI region of all of the remaining 68 samples whose haplogroups could not be identified by restriction analysis were amplified to

TABLE 2. Fragments studied for all samples not assigned to haplogroup A, B, C, D, or X

Primer coordinants	Restriction site	Haplogroup[1]	Geographic origin[1]
H 7060-7041, L 6949-6969	−7025 *Alu* I	H	Europe
H 8366-8345, L 8188-8207	+8249 *Ava* II	I	Europe
H 10107-10088, L 9911-9932	+10028 *Alu* I	I	Europe
H 12338-12309, L 12104-12124	+12308 *Hin*fI	U	Europe
H 3717-3701, L 3388-3408	+3592 *Hpa* I	L	Africa
H 10579-10557, L 10270-10290	+10397 *Alu* I	M (C, D, G & E)	Asia
	+10394 *Dde* I	I, J, K & M	Europe and Asia

[1] The haplogroups studied account for between 60 and 80% of the haplotypes in Europe, Africa and Asia. The presence of these haplogroups in Native Americans is assumed to result from recent admixture.

detect CR mutations associated with a known haplogroup.

RESULTS

Thirty of the 40 "other" samples extracted from serum (or 75%) exhibited the *Dde* I site losses at np 1715 and np 10394 and, therefore, are members of haplogroup X, as shown in Table 1. None of the remaining 10 samples exhibited a *Dde* I site loss at one, but not the other, of these two sites. These remaining 10 samples that are not members of haplogroups A, B, C, D, or X could represent European admixture, other yet unrecognized founding haplogroups, or mutations of one of the five founding haplogroups. Four (two Cherokee and two Southwestern Chippewa) of these 10 samples were members of the European haplogroup H and one (a Narrangansett) was a member of haplogroup L which is of subsaharan African origin. An insufficient sample remained to test one (a Chippewa) of the 10 "other" samples for the haplogroups listed in Table 2. Of the four remaining samples lacking the characteristic restriction sites that identify haplogroups A, B, C, D, and X, one (a Cherokee) exhibited all four of the CR mutations associated with haplogroup C, C → T transitions at np 16223 and 16327 and T → C transitions at 16298 and 16325, (the fragment containing the *Alu* I restriction site gain at np 13262 characteristic of haplogroup M, of which haplogroup C is a subset, did not amplify for this sample). In addition, a Creek sample lacking the restriction site characteristic of haplogroup A exhibited all five CR mutations typically associated with that haplogroup (C → T transitions at 16111, 16223 and 16290; T → C transitions at 16362 and 16519 and a G → A transition at 16319). Of the two remaining samples, one exhibited one of the two mutations (a T → C transition at np 16126) characteristic of European haplogroup J as well as several other mutations including a rare A → T transversion at np 16113. The last (a Chippewa) sample exhibited both of the CR mutations characteristic of European haplogroup T, C → T transitions at np 16294 and 16296.

Two of the 30 Salteaux Chippewa samples studied (but not typed for haplogroups A, B, C or D) exhibited the *Dde* I site losses at np 1715 and np 10394 confirming its presence in this northwestern Ojibwa group. Haplogroup X was present in five of the six Algonquian-speaking tribal groups studied in frequencies ranging from 7% to 40%. Our study is the first report of haplogroup X in an Algonquian tribe other than Chippewa and demonstrates its presence throughout this widely distributed language group.

Haplogroup X was also identified in both of the "other" Kiowa samples that were studied and in all three samples from Jemez Pueblo. While the frequency of haplogroup X in the Jemez Pueblo was only 8%, the Kiowa samples assigned to haplogroup X in this study were the only two of five Kiowa samples studied previously that were found not to be members of haplogroups A, B, C, or D. While this suggests that haplogroup X is very common in the Kiowa, even frequency estimates based on much larger samples can be seriously influenced by sampling error.

As shown in Figure 2, at least 9 of the 11 samples assigned to haplogroup X by restriction analysis that were sequenced exhibit C → T transitions at np 16223 and 16278 as well as two other CR mutations, an A → C

transversion at np 16183 and a T → C transition at np 16189. The T → C transition at np 16519 was present in all four of these 11 samples that were sequenced at this position, and has been reported by Brown et al. (1998) in their samples assigned to haplogroup X. In addition several mutations in the HVSII CR are also consistently present in these samples (Malhi, unpublished data). All 30 sequences in Figure 2 exhibit at least two of these four CR mutations characteristic of haplogroup X in the New World. These include European members of haplogroup X and Asian (Mongolian) members of haplogroup F. However, only two of the samples, one Turk and one Basque, share all four of these mutations with the nine Native Americans of haplogroup X.

DISCUSSION

None of the tribes in which haplogroup X was found in this study are known to be closely related to Nuu-Chah-Nulth [albeit the controversial Almosan hypothesis (Greenberg, 1987) proposes remote connections between Algonquian and Wakashan] or Yakima in whom haplogroup X had previously been reported (Forster et al., 1996). Haplogroup X has now been reported in contemporary members of seven specific unrelated language families (Athapaskan, Algonquian, Kiowa-Tanoan, Wakashan, Plateau Penutian, Northern Hokan, and Siouan) which are distributed throughout markedly noncontiguous geographic regions of the Canadian Subarctic/Great Lakes region, the Southwestern U.S., the Southern Plains and the Central and Northwest Coasts, as shown in Figure 1. As part of a separate study we identified one full-blood Sisseton-Wahpeton Sioux as a member of haplogroup X, but this sample also exhibited albumin Naskapi, suggesting that the presence of both haplogroup X and albumin Naskapi in Sioux might result from admixture with nearby Algonquian-speaking (e.g., Chippewa) groups (Smith et al., 1999). The Cheyenne, the southwestern-most of the Algonquians, might either have diverged from the main body of Proto-Algonquians after crossing the Continental Divide during their migration eastward from their original homeland on the Columbia Plateau or represent a very late southwestward movement onto the Plains after this migration reached its terminus in the region south of the Great Lakes (Denny, 1991; Goddard, 1994). The diversity between Wiyot/Yurok, the western-most relatives of Algonquian languages, and the Proto-Algonquian language, suggests that this migration began around 4,000 years B.P. (Goddard, 1975).

It is conceivable that the presence of haplogroup X in Nuu-Chah-Nulth (Nootka) and Yakima resulted from admixture with Proto-Algonquians (or visa versa) before they abandoned the Columbia Plateau to the Penutian-speaking groups whose descendants still live there. This explanation is also consistent with the presence in the Nuu-Chah-Nulth of Albumin Naskapi, which is otherwise restricted to, and pervasive throughout, all modern Algonquian and Athapascan tribes (Smith et al., 1999). The principal divergence of the central Algonquian languages probably occurred between 2,000 and 3,000 years BP in southern Ontario (Siebert, 1967), the eastern terminus of the Algonquian migration (Denny, 1991; Goddard, 1994). The alternative explanation that subsequent admixture among the Cheyenne, the Salteaux (Northwestern) Chippewa, the Lac Courte Oreilles (Southwestern) Chippewa and the Micmac, who collectively span a vast geographic range (see Fig. 1), is responsible for spreading haplogroup X from a single (e.g., mutational or recent European) origin seems less likely. Moreover, the probability of haplogroup X (whose frequency in Europe is about 3%), but not other European haplogroups, originating through recent European admixture or mutation simultaneously and independently in each of these Algonquian tribal groups and in both tribes (i.e., Kiowa and Jemez) speaking Kiowa-Tanoan, is very low. The Kiowa and Jemez Pueblo speak languages that are closely related to each other (Harrington, 1910; Campbell, 1999), sharing a common proto-language that is about 3,000 years old (Hale and Harris, 1979:171), but not demonstrably related to languages spoken by all other individuals known to represent haplogroup X (Campbell, 1997; Greenberg, 1987;

		16093	16111	16129	16145	16182	16183	16189	16191	16192	16213	16223	16227	16235	16248	16254	16255	16261	16264	16265	16278	16291	16294	16297	16309	16319	16324	16325	16357	16362	16519
	CRS	T	C	G	G	A	A	T	C	C	G	C	A	A	C	A	G	C	C	A	C	C	C	T	A	G	T	T	T	T	T
1	Kiowa 70	.	.	.	.	.	C	C	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G	.	.	.	-	-	-
2	Kiowa 50	C	.	.	.	.	C	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
3	Jemez 22	.	.	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	.	.	T	T	.	.	.	A	.	.	C	.	C
4	Jemez 435	.	.	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	.	.	T	T	.	.	.	A	.	.	C	.	C
5	Jemez 990	.	.	.	.	.	C	C	.	.	A	T	.	.	.	.	.	.	.	.	T	T	.	.	.	A	.	.	C	.	C
6	Wisconsin Chippewa 1	C	.	.	.	.	C	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
7	Micmac 4	C	.	.	.	.	C	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
8	Cheyenne/Arapaho 22	C	.	.	.	C	C	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
9	Sioux	C	.	.	.	.	C	C	.	.	-	-	-	.	.	.	.	.	G	G	T	-	-	-	-	-	-	-	-	-	-
10	Pomo (Sam) 19	.	.	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C
11	Nootka 1	.	.	.	.	.	.	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
12	Nootka 4	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
13	Blackfoot	.	.	.	.	.	C	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.
14	Navajo	.	.	.	A	.	C	C	.	.	A	T	.	.	.	C	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C
15	Yakima	.	.	.	.	.	.	C	.	.	A	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	-	-	-
16	Oneota 24	C	.	.	.	.	.	C	.	.	.	T	G	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	-
17	Brazil 01	.	.	.	.	.	.	C	.	T	.	T	T	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	-
18	Windover 94	-	-	-	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	-	-	-	-	-	-
19	European	.	.	.	.	.	.	C	T	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
20	Turkey 58	.	.	.	.	.	C	C	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	-
21	Turkey 43	.	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	T	.	.	T	.	.	.	.	.	.	.	C	.	-
22	India H17	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	-
23	Tuscany 26	.	.	.	.	.	.	C	.	.	.	T	.	.	T	.	.	.	.	.	T	.	.	.	.	.	C	.	C	.	-
24	Tuscany 28	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	.	C	.	-
25	Tuscany 34	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	C	.	-
26	Tuscany 38	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	T	.	.	.	.	.	C	C	-
27	Basque 24	.	.	.	.	.	C	C	.	.	.	T	.	.	.	.	A	.	.	.	T	.	.	.	.	.	.	.	.	.	-
28	Basque 09	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
29	Mongolian 8.22	C	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	C	-
30	Mongolian 17.4	.	.	A	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	-

Fig. 2. Comparison of Native American mtDNA lineages of 11 samples assigned to haplogroup X in this study with those in other regions. The Oneota, Brazilian and Windover sequences (numbers 16, 17 and 18) are prehistoric and dated to about 600 (Stone and Stoneking, 1998), 4,000 (Ribeiro-Dos-Santos et al., 1996) and 7,000–8,000 (Doran et al., 1986; Hauswirth et al., 1994) years BP, respectively. The modern CR sequences from Tuscany, Turkey and Mongolia are published in Francalacci et al. (1996), Comas et al. (1996), and Kolman et al. (1996), respectively, and those for the European, Indian and Basque samples appear in Vigilant et al. (1991), Mountain et al. (1995), and Bertrampetit et al. (1995). The Nootka and Yakima CR sequences, cited in Forster (1996), are given in Ward et al., (1991) and Shields et al. (1993), the Pomo sequence is described in Kaestle (1998). The Kiowa, Jemez, Chippewa, Micmac and Cheyenne Arapaho CR sequence samples are first reported in this paper and all samples are compared with the Anderson (first) sequence (Anderson et al., 1981). The Sioux CR sequence has not been previously published but its membership in haplogroup X, based on the losses of Dde I restriction sites at np 1715 and np 10394, was reported in Smith et al. (1999).

Campbell and Mithun, 1979; Sapir, 1929). Since the Kiowa and Jemez are geographically separated, they might seem to be more likely to share haplogroup X due to common ancestry than to admixture. The presence of haplogroup X in Navaho, but not Apache or any Northern Athapascans that have been studied (e.g., Dogrib or Tanana), is consistent with ethnohistorical reports that at least ten clans claiming Anasazi ancestry, at least one of which (the Coyote Pass People) originated in Jemez Pueblo, joined and intermarried with the Navaho (Brugge, 1983). However, one of the Kiowa haplotypes is identical to one of the Algonquian haplotypes, and the Navaho haplotype is more similar to the Algonquian haplotype than to any of the Jemez haplotypes. Further studies are needed to identify the source of the haplogroup X haplotypes in the American Southwest. The apparent antiquity of haplogroup X in such geographically and/or linguistically dispersed tribal groups is consistent with its status as a founding Native American matriline.

Curiously, haplogroup X is found in modern populations of Europe and Southwest Asia but not in those of Central Siberia, which is now regarded as the source for the peopling of the New World (e.g., see Karafet et al., 1999; Kolman et al., 1996; Merriwether et al., 1996; Santos et al., 1999). Several CR mutations that were found to be characteristic of haplogroup X in this study (e.g., the T → C transition at np 16189 and C → T transitions at np 16223 and np 16278) are found in some members of the Asian haplogroup F, but they are infrequent, not found together, or with the relatively rare A → C transversion at 16183, and lack the *Dde* I site loss at np 1715 (e.g., see Mongolian sequences in Table 2). Thus, it is unlikely that the haplogroups F and X are closely related. Alternatively, one of the founding haplogroups of Native America might be of European origin, in which case the provocative issues of its time and route of entry into the New World and its incorporation into Asian-derived tribal groups emerge. A more likely scenario is that haplogroup X was once found in both Europe and Asia but became extinct in Asia after Native Americans peopled the New World from some site in East Asia. This explanation is consistent with the distribution of Y-chromosome haplotype 1C from which Karafet et al. (1999) hypothesized a bifurcated migration from the Lake Baikal region of western Siberia, one branch leading to Europe and another to the New World.

Haplogroup X, with a continent-wide frequency of about 3%, is the least common of the five predominant haplogroups of Native America, but it, like the other four haplogroups, exhibits a nonrandom distribution in North America characterized by very high frequencies in some modern (e.g., Chippewa, Micmac, Kiowa) populations. As such, its distribution among modern Native American groups might provide clues to common ancestral relationships among modern tribal groups, and comparisons with ancient mtDNA might be useful for assessing ancestor/descendant relationships in North America.

In addition to confirming its legitimacy as a founding haplogroup, the widespread presence of haplogroup X in prehistoric samples will be particularly useful for assessing the antiquity of Algonquian-speaking peoples in the Subarctic/Great Lakes region and evaluating the hypothesis that the Proto-Algonquians originated on the Columbia Plateau, then migrated eastward to their present homes between three and four millennia ago (Denny, 1991; Malhi, 1998). The distribution of haplogroup X in prehistoric skeletal populations in the Southwest United States associated with Anasazi, Hohokam, Fremont, and Hakataya cultural manifestations might help sort out the relationships between each of them and Tanoan-speaking Pueblo groups living there today. Two samples each from the prehistoric Anasazi and Fremont populations were found not to exhibit the restriction sites characteristic of haplogroup A, B, C, or D (Parr et al., 1996; Carlyle, 1999) and might actually be haplogroup X, which we have shown to persist in Pueblo groups in that area. If samples from the prehistoric Windover, Florida site (Doran et al., 1985) exhibiting the C → T transition at np 16278 but lacking the restriction sites characteristic of any of the four common New World haplogroups (Hauswirth et al., 1994) are members of haplogroup X, its absence in

modern Muskogean-speaking populations in the Southeastern United States is consistent with the well-documented archaeological, ethnohistorical and linguistic evidence that they were not closely related to the prehistoric peoples that once lived there (Milanich and Fairbanks, 1980). Likewise, its relatively high frequency in Algonquians, Penutians, the Kiowa-Tanoans, and Northern Hokans suggests that their remote common ancestor might have been related to the Windover population. Other tribal groups, both modern and prehistoric, samples of which have not been screened, or screened in insufficient numbers, might also be found to carry haplogroup X.

Based on results of this study, it seems likely that modern samples assigned, in other studies, to "other" haplogroups are more likely to be members of haplogroup X than to represent recent European admixture or mutations of restriction sites characteristic of haplogroups A, C, or D. Only 6 of the 40 samples studied here, which were discovered by screening more than 1,000 Native Americans, exhibited evidence of European admixture in the matriline by belonging to haplogroups H, J, or T. If these represent roughly 70% of all modern European-American haplogroups, as in Europe, our results are consistent with a matrilineal admixture rate of less than 1% among the samples studied.

Only 2 of the 40 samples (one each a member of Native American haplogroups A and C) were mistakenly classified "other" because the restriction site characteristic of its haplogroup had been destroyed by a mutation. Thus, such mutations are unlikely to make significant contributions to errors in haplogroup assignment. Errors due to nonconcordance between the restriction site gain or loss and the CR mutations generally associated with it that are characteristic of members of a particular haplogroup can also lead to mistyping but are relatively uncommon. In addition, a conservative strategy of assigning samples to haplogroups resulted in an overestimate of the proportion of samples belonging to haplogroups other than A, B, C, or D in the study of Lorenz and Smith (1996). The two sources of errors cited above suggest that haplogroup assignments cannot be based solely on either restriction analysis or CR sequences alone, but rather require confirmation from both methods. Of particular note is the complete absence of haplogroups not assigned to A, B, C, D, or X without clear evidence of recent admixture (usually, European), although such haplogroups might exist in other geographic regions of, or at earlier time periods in, the New World.

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LITERATURE CITED

Anderson S, Bankier AT, Barrell BG, DeBruijn MHL, Coulson AR, et al. 1981. Sequence and organization of the human mitochondrial genome. Nature 290:457–465.

Bailliet G, Rothhammer F, Carnese FR, Bravi CM, Bianchi NO. 1994. Founder mitochondrial haplotypes in Amerindian populations. Am J Hum Genet 54:27–33.

Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan K, Chen K-H, Wallace DC. 1992. Southeast Asian mitochondrial DNA reveals genetic continuity of ancient Mongoloid migrations. Genetics 130:139–152.

Bertranpetit J, Sala J, Calafell F, Underhill PA, Moral P. 1995. Human mitochondrial DNA variation and the origin of Basques. Ann Hum Genet 59:63–81.

Bonatto SL, Salzano FM. 1997a. A single and early migration for the peopling of the Americas supported by mitochondrial DNA sequence data. Proc Nat Acad Sci USA 94:1866–1871.

Bonatto SL, Salzano FM. 1997b. Diversity and age of the four major mtDNA haplogroups, and their implications for the peopling of the New World. Am J Hum Genet 61:1413–1423.

Brown KS, Hanna BL, Dahlberg AA, Strandskov HH. 1958. The distribution of blood group alleles among Indians of southwest North America. Am J Hum Genet 10:175–195.

Brown MD, Schurr TG, Husseini S, Wallace DC. 1997. The nature of mtDNA variation in Native Americans: founding lineages "other" haplotypes and genetic diversity of ancestral populations. Am J Phys Anthropol 24 (suppl):83–84.

Brown MD, Hosseini SH, Torroni A, Bandelt H-J, Allen JC, Schurr TG, Scozzari R, Cruciani F, Wallace DC. 1998. mtDNA haplogroup X: An ancient link between Europe/Western Asia and North America. Am J Hum Genet 63:1852–1861.

Brugge DM. 1983. Navajo. In: Ortiz A, Sturtevant, editors. Handbook of North American Indians, Southwest, Vol. 10. Washington, D.C.: Smithsonian Institution.

Campbell L. 1997. American Indian languages: The historical linguistics of Native America. New York: Oxford University Press.

Campbell L, Mithun M. 1979. North American Indian historical linguistics in current perspective. In: Campbell L, Mithun M, editors. The languages of Native America: A historical and comparative assessment. Austin: University of Texas Press. p 3–69.

Carlyle SW, Parr RL, Hayes MG, O'Rourke DH. 1999. The context of maternal lineages in the greater Southwest. Am J Phys Anthropol (in press).

Comas D, Calafell F, Mateu E, Perez-Lezaun A, Bertranpetit J. 1996. Geographic variation in human mitochondrial DNA control region sequence: The population history of Turkey and its relationship to the European populations. Mol Biol Evol 13:1067–1077.

Denny JP. 1991. The Algonquian migration from Plateau to Midwest: Linguistics and archaeology. Papers of the 22nd Algonquian Conference. Carleton, Ottawa: Carleton University Press.

Doran GH, Dickel DN, Ballinger WE, Jr, Agee OF, Lapis PJ, Hauswirth WW. 1986. Anatomical, cellular and molecular analysis of 8,000-yr-old human brain tissue from the Windover Archaeological Site. Nature 323: 803–806.

Driver HE, Massey WC. 1957. Comparative studies of North American Indians. Trans Am Phil Soc XLVII: 165–456.

Easton RD, Merriwether DA, Crews DE, Ferrell RE. 1996. mtDNA variation in the Yanomami: Evidence for additional New-World founding lineages. Am J Hum Genet 59:213–225.

Forster P, Harding R, Torroni A, Bandelt H-J. 1996. Origin and evolution of Native American mtDNA variation: A reappraisal. Am J Hum Genet 59:935–945.

Francalacci P, Bertranpetit J, Calafell F, Underhill PA. 1996. Sequence diversity of the control region of mitochondrial DNA in Tuscany and its implications for the peopling of Europe. Am J Phys Anthropol 100:443–460.

Goddard I. 1975. Algonquian, Wiyot, and Yurok: proving a distant genetic relationship. In: Kinkade MD, Hale KL, Werner O, editors. Linguistics and anthropology: In honor of C.F. Vogelin. Lisse: Peter de Ridder Press. p 249–262.

Goddard I. 1994. The west-to-east cline in Algonkian dialectology. In: Cowan W, editor. Actes du Vingt-Cinquième Congrès des Algonquinistes. Carleton, Ottowa: Carleton University Press. p 187–211.

Greenberg JH. 1987. Language of the Americas. Stanford: Stanford University Press.

Hale K, Harris D. 1979. Historical linguistics and archaeology. In: Ortiz A, editor. Handbook of North American Indians, Southwest, Vol 9. Washington, D.C.: Smithsonian Institution. p 170–177.

Harrington JP. 1910. On phonetic and lexic resemblances between Kiowan and Tanoan. Am Anthropol 12:119–123 (also in Papers of the School of American Archaeology 12, Santa Fe, New Mexico).

Hauswirth WW, Dickel CD, Rowold DJ, Hauswirth MA. 1994. Inter- and intrapopulation studies of ancient humans. Experientia 50:585–591.

Horai S, Kondo R, Nakagawa-Hattori Y, Hayashi S, Sonoda S, Tajima K. 1993. Peopling of the Americas founded by four major lineages of mitochondrial DNA. Mol Biol Evol 10:23–47.

Kaestle F. 1997. Molecular archaeology: an analysis of ancient Native American DNA from western Nevada. Nev Hist Soc Quart 40:85–96.

Kaestle F. 1998. Molecular evidence for prehistoric Native American population movement: The numic expansion. Ph.D. dissertation. University of California, Davis.

Karafet TM, Zegura SL, Posukh O, Osipova L, Bergen A, Long J, Goldman D, Klitz W, Harihara S, Knijff P, Wiebe V, Griffiths RC, Templeton AR, Hammer MF. 1999. Ancestral Asian source(s) of New World Y-chromosome founder haplotypes. Am J Hum Genet 64:817–831.

Kolman CJ, Sambuughin N, Bermingham E. 1996. Mitochondrial DNA analysis of Mongolian populations and implications for the origin of New World founders. Genetics 142:1321–1334.

Lorenz JG, Smith DG. 1996. Distribution of four founding mtDNA haplogroups among Native North Americans. Am J Phys Anthropol 101:307–323.

Lorenz JG, Smith DG. 1997. Distribution of sequence variations in the mtDNA control region of Native North Americans. Hum Biol 69:749–776.

Malhi RS. 1998. The genetic prehistory of the Columbia Plateau. Paper presented at the annual meetings of the Society of American Archaeologists. Seattle, WA.

Merriwether DA, Ferrell RE. 1996. The four founding lineage hypothesis for the New World; a critical reevaluation. Mol Phylogenet Evol 5:241–246.

Merriwether DA, Rothhammer F, Ferrell RE. 1995. Distribution of the four founding lineage haplotypes in Native Americans suggests a single wave of migration for the New World. Am J Phys Anthropol 98:411–430.

Merriwether DA, Hall WW, Vahlne A, Ferrell RE. 1996. mtDNA variation indicates Mongolia may have been the source for the founding population for the New World. Am J Hum Genet 59:204–212.

Milanich J, Fairbanks C. 1980. Florida archaeology. New York: Academic Press.

Mountain J, Hebert JM, Bhattacharyya S, Underhill PA, Ottolenghi C, Gadgil M, Cavalli-Sforza LL. 1995. Demographic history of India and mtDNA-sequence diversity. Am J Hum Genet 56:979–992.

Parr RL, Carlyle SW, O'Rourke DH. 1996. Ancient DNA analysis of Fremont Amerindians of the Great Salt Lake wetlands. Am J Phys Anthropol 99:507–518.

Ribeiro-dos-Santos AKC, Santos SEB, Machado AL, Guapindaia V, Zago MA. 1996. Heterogeneity of mitochondrial DNA haplotypes in pre-Columbian natives of the Amazon region. Am J Phys Anthropol 101:29–37.

Santos FR, Pandya A, Tyler-Smith C, Pena SDJ, Schanfield M, Leonard WR, Osipova L, Crawford MH, Mitchell RJ. 1999. The central Siberian origin for Native American Y chromosomes. Am J Hum Genet 64:619–628.

Sapir E. 1929. Central and North American languages. Encyclopedia Britannica (14th edition). Vol. 5. New York: Encyclopedia Britanica Company. p 138–141.

Schurr TG, Ballinger SW, Gan Y-Y, Hodge JA, Merriwether DA, Lawrence DN, Knowler WC, Weiss KM, Wallace DC. 1990. Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. Am J Hum Genet 46:613–623.

Scozzari R, Cruciani F, Santolamazza P, Sellitto D, Cole DEC, Rubin LA, Labuda D, Marini E, Succa V, Vona G, Torroni A. 1997. mtDNA and Y chromosome-specific polymorphisms in modern Ojibwa: Implications about the origin of their gene pool. Am J Hum Genet 60:241–244.

Shields GF, Schinicchan AM, Frazier BL, Redd A, Voevoda MI, Reed JK, Ward RH. 1993. mtDNA sequences suggest a recent evolutionary divergence for Beringian and northern North American populations. Am J Hum Genet 53:549–562.

Siebert FT Jr. 1967. The original home of the proto-Algonquian people. Anthropol Ser 78. Natl Mus Can Bull 214:13–47.

Smith DG, Lorenz J, Rolfs BK, Bettinger RL. 1999. Implications of the distribution of Albumin Naskapi and Albumin Mexico for New World prehistory. Am J Phys Anthropol (in press).

Stone AC, Stoneking M. 1993. Ancient DNA from a pre-Columbian Amerindian population. Am J Phys Anthropol 92:463–471.

Stone AC, Stoneking M. 1998. mtDNA analysis of a prehistoric Oneota population: Implications for the peopling of the New World. Am J Hum Genet 62:1153–1170.

Torroni A, Wallace DC. 1995. Mitochondrial DNA haplogroups in Native Americans. Am J Hum Genet 56:1234–1236.

Torroni A, Schurr TG, Yang C-C, Szathmary EJE, Williams RC, Schanfield MS, Troup GA, Knowler WC, Lawrence DN, Weiss KM, Wallace DC. 1992. Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130:153–162.

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, Vullo CM, Wallace DC. 1993. Asian affinities and continental radiation of the four founding Native American mtDNAs. Am J Hum Genet 53:563–590.

Torroni A, Lott MT, Cabell MF, Chen Y-S, Lavergne L, Wallace DC. 1994. mtDNA and the origin of Caucasians: Identification of ancient Caucasian-specific haplogroups, one of which is prone to a recurrent somatic duplication in the D-Loop region. Am J Hum Genet 55:760–776.

Torroni A, Huoponen K, Francalacci P, Petrozzi M, Morelli L, Scozzari R, Obinu D, Savontaus M-L, Wallace DC. 1996. Classification of European mtDNAs from an analysis of three European populations. Genetics 144:1835–1850.

Vigilant L, Stoneking M, Harpending H, Hawkes K, Wilson AC. 1991. African populations and the evolution of human mitochondrial DNA. Science 253:1503–1507.

Wallace DC, Garrison K, Knowler WC. 1985. Dramatic founder effects in Amerindian mitochondrial DNAs. Am J Phys Anthropol 68:149–155.

Ward RH, Frazier BL, Dew-Jager K, Paabo S. 1991. Extensive mitochondrial diversity within a single Amerindian tribe. Proc Natl Acad Sci USA 88:8720–8724.